Amendments to the Claims

1. (Currently amended) A method of screening an agent to determine its usefulness in treating a condition characterised by pancreatic islet or β-cell dysfunction, the method comprising:

(a) ~~establishing a paradigm in which at least one protein is differentially expressed in relevant tissue from, or representative of, subjects having differential levels of pancreatic islet or β-cell function;~~identifying proteins which are differentially expressed in biological samples obtained from subjects having reduced or increased pancreatic islet or β-cell function and normal subjects, said samples being obtained before and after treatment of said subjects with a compound which alleviates or improves pancreatic islet or β-cell dysfunction;

(b) providing ~~obtaining~~ a biological sample of ~~relevant~~ tissue or cells which undergo a biological change in response to the action of insulin; ~~taken from, or representative of, a subject having reduced pancreatic islet or β-cell function, who or which has been treated with the agent being screened~~;

(c) contacting the sample of step b) with said agent and identifying proteins which are differentially expressed in response to said agent;~~determining the presence, absence or degree of expression of the differentially expressed protein or proteins in the tissue from, or representative of, the treated subject~~; and,

(d) comparing the results of a) and c) thereby identifying those agents which alter the expression levels of said proteins towards that of a subject having substantially normal pancreatic islet or β cell function ~~selecting or rejecting the agent according to the extent to which it changes the expression, activity or amount of the differentially expressed protein or proteins in the treated subject having reduced pancreatic islet or β-cell function~~.

2-3. (Cancelled)

4. (Previously presented) The method of claim 1, wherein the pancreatic islet or β-cell dysfunction is a result of a disorder which causes a reduction in pancreatic islet or β-cell mass and/or a reduction in a pancreatic islet or β-cell biological activity.

5. (Currently amended) The method of claim 1, wherein ~~the paradigm is based on tissue from~~ said subjects having reduced or increased pancreatic islet or β-cell function are non-insulin dependent diabetic subjects ~~and normal subjects~~.

6. (Currently amended) The method of claim 1, wherein the said sample comprises ~~relevant tissue is~~ pancreatic islets.

7-8. (Cancelled)

9. (Currently amended) The method of claim 1 ~~8~~, wherein the differential levels of protein expression are not observed in normal subjects who have and have not been treated with the agent.

10. (Cancelled)

11. (Currently amended) The method of claim 1 ~~10~~, wherein the differential levels of protein expression are not observed in normal subjects and subjects having reduced pancreatic islet or β-cell function, both groups of subject being untreated with the agent.

12. (Currently amended) The method of claim 1, wherein the ~~paradigm is based on animals which are models of~~ subjects having reduced or increased pancreatic islet or β-cell

function are animals which have non-insulin dependent diabetes as a result of a genetic mutation, said animals being selected from ~~such as~~ ob/ob, db/db, agouti, fat, and fa/fa mice ~~together with~~ lean ~~littermates~~.

13. (Currently amended) The method of claim 1, wherein said reduced or increased pancreatic islet or β-cell function ~~the paradigm is based on animals in which islet or β-cell dysfunction~~ is exacerbated by dietary treatment.

14. (Currently amended) The method of claim 1, wherein said subjects having reduced or increased pancreatic islet or β-cell function are ~~the paradigm is based on~~ the offspring of pregnant animals fed on a reduced protein diet.

15. (Original) The method of claim 14, wherein the diet fed to the offspring post weaning is additionally a high fat diet.

16. (Currently amended) The method of claim 1, wherein said subjects having reduced or increased pancreatic islet or β-cell function are ~~the paradigm is based on~~ desert rodents selected from the group consisting of ~~such as~~ spiny mice ~~or~~ and sand rats which develop diabetes on normal laboratory diets but remain normoglycaemic on their natural diet.

17. (Currently amended) The method of claim 1, wherein said subjects having reduced or increased pancreatic islet or β-cell function are animals which have ~~the paradigm is based on animals with~~ gender selective differences in pancreatic islet or β-cell mass.

18. (Currently amended) The method of claim 1, wherein the differential protein expression is compared in ~~paradigm is based on~~ closely related animals, such as C57BI/6 and C57BI/Ks mice which show differences in pancreatic islet or β-cell mass

in response to said treatment.

19. (Currently amended) The method of claim 4 ~~1~~, wherein differential levels of islet cell or β-cell mass or function are induced by modifying the diet of pregnant animals or by comparing pregnant and non-pregnant animals ~~in the paradigm~~.

20. (Currently amended) The method of claim 1, wherein ~~in the paradigm,~~ the subjects have ~~having differential levels of pancreatic islet or β-cell function comprise normal subjects and subjects having r~~educed levels of pancreatic islet or β-cell function.

21. (Currently amended) The method of claim 20, wherein said reduced pancreatic islet or β-cell function is ~~the reduced levels of pancreatic islet or β-cell function are~~ the result of non-insulin dependent diabetes (type 2 diabetes), syndrome X (insulin resistance syndrome) or gestational diabetes.

22. (Currently amended) The method of claim 1, wherein ~~in~~ the ~~paradigm, the subjects having differential levels of pancreatic islet or β-cell function comprise normal subjects and~~ subjects ~~having~~ have a higher than normal level of pancreatic islet or β-cell function.

23. (Original) The method of claim 22, wherein the higher levels of pancreatic islet or β-cell function in the subjects are obtained by treatment with an insulin sensitiser drug, dietary restriction or exercise.

24. (Original) The method of claim 23, wherein the insulin sensitising drug is thiazolidinedione insulin sensitiser.

25. (Original) The method of claim 24, wherein the thiazolidinedione insulin sensitiser is rosiglitazone (BRL

49653).

26. (Original) The method of claim 23, wherein the insulin sensitiser drug is a non-thiazolidinedione acting as an agonist or partial agonist of the PPAR gamma nuclear receptor.

27. (Original) The method of claim 23, wherein the insulin sensitiser drug is a 3-adrenoceptor agonist or leptin.

28. (Original) The method of claim 22, wherein the subjects having a higher than normal level of pancreatic islet or β-cell function are pregnant animals.

29. (Original) The method of claim 22, wherein the higher level of pancreatic islet or β-cell function in the subjects are obtained by administration of an insulin secretagogue peptide or drug.

30. (Original) The method of claim 29, wherein the insulin secretagogue is GLP-1 or a stable GLP-1 analogue or exendin 4.

31. (Previously presented) The method of claim 23, wherein the insulin secretagogue further stimulates insulin production and/or the genesis of islet cells.

32. (Currently amended) The method of claim 1, wherein ~~the paradigm~~ said differential expression is established using two-dimensional gel electrophoresis carried out on said biological samples ~~the relevant tissue or a protein-containing extract thereof~~.

33. (Previously presented) The method of claim 1, further comprising the step of isolating a differentially expressed protein identified in the method.

34. (Original) The method of claim 33, further comprising the step of characterising the isolated protein.

35. (Previously presented) The method of claim 1, wherein the differentially expressed protein or proteins comprise at least one of POM6, POM7 POM8, POM9, POM10, POMT1, POMT2, POMT3, POMT4, POMT5, POMT11, POMT12, POMT13, PSEM14 AND PSEM15.

36. (Original) The method of claim 34, further comprising using the protein in an assay for specific binding partners of the protein.

37. (Original) The method of claim 34, further comprising using the protein in an assay to screen for agonists or antagonists of the protein.

38. (Previously presented) The method of claim 1, wherein the agents or proteins are screened using a high through put screening method.

39. (Previously presented) A method of making a pharmaceutical composition which comprises having identified an agent using the method of claim 1, the further step of manufacturing the agent and formulating it with an acceptable carrier to provide the pharmaceutical composition.

40-43 Cancelled

44. (Previously presented) A method of treating a condition characterised by islet or β-cell dysfunction in a patient, the method comprising administering to the patient a therapeutically or prophylactically effective amount of an agent identified by a method of claim 1.

45. (Original) The method of claim 44, wherein the pancreatic islet or P-cell dysfunction is a result of non-insulin dependent diabetes or type 2 diabetes, syndrome X or insulin resistance syndrome or gestational diabetes.

46. (Cancelled)

47. (Currently amended) The method of claim 1 ~~46~~, wherein the sample is a tissue sample or body fluid sample or urine.

48. (Currently amended) The method of claim 1 ~~47, wherein in the paradigm~~ at least four proteins are identified as differentially expressed in said subjects having reduced or increased pancreatic islet or β cell function thereby~~,~~ providing a multi-protein fingerprint ~~of the nature or degree~~ of the pancreatic islet or β-cell dysfunction.

49. (Cancelled)

50. (Original) A method of treatment by the use of an agent that will restore the expression of one or more differentially expressed proteins in the pancreatic islet or β-cell dysfunction state to that found in the normal state in order to prevent the development of non-insulin dependent diabetes in a pre-diabetic subject.

51. (Original) A method whereby the pattern of differentially expressed proteins in a tissue sample or body fluid sample or urine of an individual with pancreatic islet or β-cell dysfunction is used to predict the most appropriate and effective therapy to alleviate the pancreatic islet or β-cell dysfunction state and to monitor the success of that treatment.

52. (Original) The method of claim 51 whereby the pancreatic islet or β-cell dysfunction state is non-insulin dependent diabetes or type 2 diabetes.

53. (Original) A protein which is differentially expressed in relevant tissue from, or representative of subjects having differential levels of pancreatic islet or β-cell dysfunction and which is as obtainable by the method of two-dimensional gel electrophoresis carried out on said tissue or a protein-containing extract thereof, the method comprising:

(a) providing non-linear immobilized pH gradient (IPG) strips of acrylamide polymer 3 mm x 180 mm;

(b) rehydrating the IPG strips in a cassette containing 25 ml. of an aqueous solution of urea (8M), 3-[(cholamidopropyl) dimethylammonio]-1-propanesulphonate (CHAPS, 2% w/v), dithioerythritol (DTE, lOmM), mixture of acids and bases of pH 3.5 to 10 (2% w/v) and a trace of Bromophenol Blue ;

(c) emptying the cassette of liquid, transferring the strips to an electrophoretic tray fitted with humid electrode wicks, electrodes and sample cups, covering the strips and cups with low viscosity paraffin oil;

(d) applying 200 micrograms of an aqueous solution of dried, powdered material of the relevant body tissue in urea (8M), CHAPS (4% w/v), Tris (40 mM), DTE (65 mM), SDS (0.05% w/v) and a trace of Bromophenol Blue to the sample cups, at the cathodic end of the IPG strips;

(e) carrying out isoelectric focusing on the gel at a voltage which increases linearly from 300 to 3500 V during 3 hours, followed by another 3 hours at 3500 V, and thereafter at 5000V for a time effective to enable the proteins to migrate in the strips to their pI- dependent final positions;

(f) equilibrating the strips within the tray with 100 ml of an aqueous solution containing Tris-HCl (50 mM) pH 6.8,

urea (6M), glycerol (30% v/v), SDS (2% w/v) and DTE (2% w/v) for 12 minutes;

(g) replacing this solution by 100 ml. of an aqueous solution containing Tris-HCl (50 mM) pH 6.8, urea (6M), glycerol (30% v/v), SDS (2% w/v), iodoacetamide (2.5% w/v) and a trace of Bromophenol Blue for 5 minutes;

(h) providing a vertical gradient slab gel 160 x 200 x 1.5 mm of acrylamide/piperazine-diacrylyl cross- linker (9-16% T/2. 6% C), polymerised in the presence of TEMED (0.5% w/v), ammonium persulphate (0.1% w/v) and sodium thiosulphate (5 mM), in Tris-HCl (0.375M) pH 8.8 as leading buffer;

(i) over-layering the gel with sec-butanol for about 2 hours, removing the overlay and replacing it with water ;

(j) cutting the IPG gel strips to a size suitable for the second dimensional electrophoresis, removing 6 mm from the anode end and 14 mm from the cathode end;

(k) over-layering the slab gel with an aqueous solution of agarose (0.5% w/v) and Tris-glycine-SDS (25 mM-198 mM- 0.1% w/v) as leading buffer, heated to 70°C and loading the IPG gel strips onto the slab gel through this over- layered solution;

(l) running the second dimensional electrophoresis at a constant current of 40 mA at 8-12°C for 5 hours; and

(m) washing the gel.

54. (Original) The protein of claim 53, wherein the protein is selected from POM6, POM7, POM8, POM9, POM10, POMT1, POMT2, POMT3, POMT4, POMT5, POMT11, POMT12, POMT13, PSEM14 AND PSEM15.

55. (Original) A differentially expressed protein having one or more of the identifying characteristics as set out in Table 2.

56. (Original) The protein of claim 55, wherein the identifying characteristics are pI and Mw.

57. (Previously presented) The method of claim 44, wherein said agent is a protein selected from POM6, POM7, POM8, POM9, POM10, POMT1, POMT2, POMT3, POMT4, POMT5, POMT11, POMT12, POMT13, PSEM14 and PSEM15.